Rec'd 2.3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	:	**Confirmation No. 7229**
Jean-Marie SAINT-REMY et al.	:	Docket No. 01699/P.UCB.09/US
Serial No. 09/362,731	:	Group Art Unit 1644
Filed July 29, 1999	:	Examiner P. Huynh
COMPOUND AND METHOD FOR THE PREVENTION AND/OR THE TREATMENT OF ALLERGY	:	

DECLARATION UNDER 37 C.F.R. 1.132

Assistant Commissioner for Patents,
Washington, D.C. 20231

Sir:

I, Jean-Marie SAINT-REMY, the undersigned, residing at Rue du Lambais 79, B-1390 Grez-Doiceau, Belgium, do hereby declare:

1. That I am a co-inventor of the above-identified application.

2. That in order to show the patentability of the above-identified application and demonstrate that there is sufficient guidance and working examples in the specification to teach one skilled in the art how to make, use and be in possession of the claimed invention, I have under my control and direction conducted the following experiments. The particulars and results of the experiments are set forth hereinbelow.

EXPERIMENTS

Example 1

Injection of a plasmid containing a tetanus toxoid (TT)-derived T cell epitope and a Der p 2-derived B cell epitope is efficient at boosting the immune response towards the allergen

The Der p 2-derived B cell epitope, p21-35, contains a T cell epitope, which is dependent on the presence of an Ile residue in position 28. Substitution of Ile28 by Asn completely abrogates the T cell activating properties of p21-35 in the BALB/c mouse. Experiments were therefore carried out with the p21-35(Ile28Asn) mutant.

To demonstrate that injection of a DNA construct made of a plasmid vector in which the DNA sequence for the TT 830-844 peptide and the p21-35(Ile28Asn) peptide from the allergen Der p 2 represented an efficient immunization method, groups of 6 BALB/c mice were injected with Der p 2, followed by IM injection of the plasmid containing either the sequence coding for TT and p21-35(Ile28Asn) (full insert plasmid) or none of such sequences ("naked" plasmid).

The sequence of the full insert is:

ATG GAT CAG TAT ATA AAA GCA AAT TCT AAA TTT ATA GGT ATA ACT GAA CTA GGA GGT TGC CAT GGT TCA GAA CCA TGT AAC ATT CAT CGT GGT AAA CCA TTC GGC GGT TGT CAC GGA AGT GAG CCT TGC AAT ATA CAC AGA GGA AAG CCG TTC TAA (Sequence ID NO. 16)

A final injection of full-length Der p 2 was then carried out. All mice had been primed with one injection of the 830-844 TT peptide10 days before the start of the immunization procedure.

Figure 1A shows the evolution of specific anti-p21-35 IgG antibodies over time in the two groups of mice. It can be seen that in the group of mice receiving the full insert plasmid a sharp increase in the level of specific antibodies is observed (day 88), while no change are seen in the "naked" plasmid treated group. This indicates that injection of the TT- p21-35(Ile28Asn) construct is very efficient at boosting the immune response towards the allergen.

In the same experiment, an additional group of 6 BALB/c mice was included in which no TT priming was applied prior to the start of the immunization procedure, which involved the full insert plasmid. Figure 1B shows that in the absence of TT priming, the TT- p21-35(Ile28Asn) DNA has no effect on the production of specific antibodies. This clearly demonstrates that recognition of the TT-derived T cell epitope is both sufficient and required for the elicitation of antibodies towards the p21-35 B cell epitope.

These data confirm that the peptide made of the 830-844 T cell epitope derived from tetanus toxoid and of the 21-35(Ile28Asn) B cell epitope of Der p 2 is truly immunogenic. It further indicates that T cells activated by recognition of tetanus toxoid-derived T cell epitope efficiently collaborate with B cells for the production of antibodies towards p21-35.

Example 2

Injection of the construct as in example 1 preferentially increases the production of anti-Der p 2 antibodies of the IgG2a isotype

In the mouse, the production of IgG2a antibodies is considered to be a marker of activation

of T helper cells belonging to the Th1 subtype, while the production of IgG1 antibodies represents a Th2-like activation (also responsible for the production of IgE antibodies).

To establish whether or not injection of the DNA construct used in example 1 could modify the proportion of IgG2a antibodies over that of IgG1, 2 groups of 6 BALB/c mice were immunized sequentially with either Der p 2, the full insert plasmid and Der p 2, or with Der p 2, the "naked" plasmid and Der p 2. The plasmid and the insert were as in Example 1. The serum of such mice was tested for the presence of Der p 2 specific IgG1 or IgG2a antibodies.

It can be seen from Figure 2 that in mice receiving the full insert plasmid, there was no change in the level of specific IgG1 antibodies, while the level of IgG2a antibodies was increased by 4-fold, as compared to the control group injected with the "naked" plasmid. These data clearly show that injection of the DNA construct alters the distribution of antibody isotypes, with a significant increase only in IgG2a antibodies, indicating a Th1-driven immune response.

Example 3

Synthetic peptides containing the TT-derived T cell epitope and the Der p 2 –derived B cell epitope (p21-35(Ile28Asn)) are efficient at inducing a specific immune response made of antibodies that cross-react with native allergen

To determine whether antibodies could be generated towards a synthetic peptide and whether such antibodies could cross-react with the corresponding region present in the native allergen molecule, mice were injected with a 32 amino-acid peptide containing the 830-844 aminoacid

sequence of tetanus toxoid linked by 2 glycine residues to the p21-35(Ile28Asn) sequence derived from Der p 2 (sequence ID NO. 5).

In this experiment, 6 BALB/c mice were primed with TT, followed 10 days later by 3 sc injections of the synthetic peptide (10 μg per injection). The serum of all these mice contained IgG antibodies directed towards p21-35, which also recognized the full-length Der p 2 allergen, as shown in Figure 3. This clearly demonstrates that antibodies generated by peptide immunization can recognize the corresponding region in the full-length allergen, thereby confirming the relevance of anti-peptide antibodies for the therapy of natural sensitization to allergen.

Example 4

The p21-35 peptide contains a T cell epitope recognized by Der p 2-sensitized patients, which is eliminated upon single substitution of Ile28

One of the preferred characteristics of the allergen-derived peptide of the present invention is that the peptide does not retain a T cell epitope, or that a significant reduction of T cell activation can be obtained.

Thus, peripheral blood was taken from a number of unrelated individuals allergic to Der p 2. Dendritic cells were derived from monocytes by methods known in the art. CD4+ T cells were obtained by negative selection using magnetic beads. Irradiated dendritic cells were incubated for 24 h in the presence of p21-35 or p21-35(Ile28Asn), and then washed before addition of autologous CD4+ T cells. After a further incubation of 4 days, 3H-thymidine was added and incorporation of the marker in T cell DNA was read as an index of activation.

Figure 4 shows representative data for 3 Der p 2 sensitive individuals. The results show that substitution of Ile28 by Asn almost completely abrogates the capacity of the p21-35 peptide to activate T cells.

To ensure that such a substitution had not altered the capacity of the peptide to be recognized by antibodies produced by non-atopic individuals, the p21-35 and p21-35(Ile28Asn) peptides were used to coat microtitration plates. Dilutions of serum obtained from non-atopic individuals were applied to such plates and the binding of antibodies was detected by addition of appropriate antiserums. Data (not shown) indicate that antibody binding to p21-35(Ile28Asn) was on average 50% stronger than binding on p21-35, indicating that Ile28 exerted a negative effect on antibody binding.

It was therefore concluded that substitution of Ile28 by Asp in the p21-35 peptide resulted in both the loss of capacity to activate specific T cells and in an increase in binding capacity for antibodies.

3. These examples confirm the immunogenicity of the construction made from a B cell epitope from an allergen linked to a T cell epitope from a different origin. This immunogenicity can be demonstrated both for DNA immunization as well as for synthetic peptides, with a preferential increase in IgG2a antibodies in the first case. Further, the data indicate that findings similar to those obtained in the mouse can be observed in Der p 2 allergic individuals.

4. I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these

statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Date: 94.05.02

Jean-Marie SAINT-REMY
(Name of Declarant)